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Elements of molecular machinery of GABAergic signaling in the vertebrate cholinergic neuromuscular junction

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ABSTRACT

It is generally accepted that gamma-aminobutyric acid (GABA) is a signaling molecule abundant in central synapses. In a number of studies though, it has been shown that GABA signaling functions in the peripheral nervous system as well, in particular, in the synapses of sympathetic ganglia. However, there exists no firm evidence on the presence of GABAergic signaling cascade in the intercellular junctions of the somatic nerve system.

By the use of immunohistochemistry methods, in the synaptic area of cholinergic neuromuscular contact in rat diaphragm, we have detected glutamate decarboxylase, the enzyme involved in synthesis of GABA, molecules of GABA, and also GAT-2, a protein responsible for transmembrane transport of GABA. Earlier we have also shown that metabotropic $GABA_B$ receptors have overlapping localization in the same compartment. Moreover, activation of $GABA_B$ receptors affects the intensity of acetylcholine release. These data taken together, allows us to suggest that in the mammalian cholinergic neuromuscular junction, GABA is synthesized and performs certain synaptic signaling function.

1. Introduction

γ-Aminobutyric acid (GABA) is generally considered to be a major inhibitory neurotransmitter in synapses of central nervous system, where it plays an important role in development, maturation and functioning of adult brain (Watanabe et al., 2002; Obata, 2013). GABA molecules are formed by decarboxylation of glutamate which is catalyzed by the enzyme L-glutamic acid decarboxylase (GAD) (EC 4.1.1.15) and subsequently transported into synaptic vesicles by vesicular GABA transporter (VGAT) (Omote and Moriyama, 2013). The synaptic action of GABA occurs via activation of ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors (Bowery et al., 2002; Olsen and Sieghart, 2008) and is terminated by uptake of GABA into neurons and glial cells. At present, the following proteins have been identified that are capable of transporting GABA molecules through membrane and thus enabling the uptake of neurotransmitter: GABA transporters 1–3 (GAT1, GAT2, GAT3) and betaine-GABA transporter (BGT1) (Zhou and Danbolt, 2013). Noteworthy, that under certain conditions, transportation process can be reversed and GABA can be released to extracellular space (Attwell et al., 1993).

Based on numerous studies carried out in the end of the 20th century, data were collected indicating that GABA may perform signaling function in peripheral nervous system as well (Watanabe et al., 2002). In particular, various elements of GABAergic signaling cascade (GABA itself, GAD, GABA receptors and transporters) were found in gastrointestinal tract, glands (thyroid, adrenal, salivary) and sympathetic ganglia, and in some preparations Ca^{2+}-dependent and tetrodotoxin sensitive mechanism of stimulus-evoked GABA release was identified in neuronal components of the tissues (Jessen et al., 1983; Tanaka, 1985). By the methods of molecular biology and genetics it has been discovered that γ2 and α1-α5 subunits of $GABA_A$ receptors are expressed in neurons of both myenteric and submucosal plexuses (Seifi et al., 2014). It has been also shown that α4 и β2/3 subunits of $GABA_A$ receptors are present in cholinergic neurons of sympathetic ganglia (Park et al., 2006; Elinos et al., 2016). Also, the neurons of the myenteric plexus were found to express both subunits, $GABA_{B1}$ and $GABA_{B2}$, of metabotropic receptor (Torashima et al., 2009). All these findings relate to the vegetative division of the peripheral nervous system, while the data on the presence of GABAergic signaling system in the somatic division are scarce. Immunoreactivity to GAD has been found in human

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